Immunoreactivity of Ubiquitin in human prostate gland

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Abstract

BACKGROUND: Ubiquitin is a low molecular weight protein which has been detected in a variety of normal and cancerous tissues. It is involved in many regulatory processes including protein proteolysis. It has been implicated in tumor pathogenesis. The role of ubiquitin in human prostate gland is investigated in this study.

METHODS: In this study, we utilized immunohistochemistry technique to localize ubiquitin in human prostate gland and correlate it with different pathological conditions of the prostate.

RESULTS: Ubiquitin was localized in normal, benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma with variations in the distribution and intensity. In BPH, ubiquitin immunoreactivity was localized mainly in the nuclei while in the adenocarcinoma was localized mainly in the cytoplasm.

CONCLUSION: The presence of ubiquitin mainly in the nuclei and in the cytoplasm of BPH and prostatic adenocarcinoma, respectively, may suggest a role of ubiquitin in the development of the above mentioned conditions. Ubiquitin could be used as a potential marker for the diagnosis and prognosis of pathological conditions of the prostate.
Introduction

Ubiquitin (Ub) is a low molecular weight protein of 8.6 KD consisting of 76 amino acids and it was found in all eukaryotic cells tested [27]. It exists in cells either freely or joined covalently to a variety of cytoplasmic, nuclear and integral membrane proteins [14,17]. Ubiquitin has been proved to act as a cofactor in ATP dependent proteolysis [11]. Since then, many studies have shown that Ub is a multifunctional regulatory protein implicated in cell cycle regulation [32], DNA repair, membrane transport [4], and signal transduction [10,37], as well as in non-lysosomal protein degradation [27].

Ubiquitin has also been implicated in the pathogenesis of several disease states through either its involvement in protein degradation or its role as stress protein in the cell [21,26]. The Ub proteolytic system may be involved in cell cycle progression, since it selectively degrades nuclear proteins such as P53, N-myc and C-myc [3]. Normally, P53 appears to function as a tumor suppressor and its degradation by Ub may result in neoplastic transformation [7,15]. The role of Ub in the genital tract has been suggested. Ubiquitin was detected in human seminal plasma [19] and in the epididymal epithelium of both rat [22] and human testes [9]. Development of mature spermatozoa involves mitochondrial degradation by ubiquitylation and destroyed by proteasome machinery of the fertilized egg [34].

Immunocytochemistry utilizing anti-ubiquitin antibodies has been used to demonstrate the presence of Ub in various normal tissues such as: lung bronchial epithelium, bile duct and collecting tubules of the kidney. In addition, Ub has been detected in malignant tumors such as: lung, liver, pancreas and stomach tumors [12]. More recently, Lombardo et al have demonstrated the presence of Ub in human benign prostatic hypertrophy (BPH) utilizing SDS-PAGE technique [20].

In addition, previous studies have demonstrated the presence of Ub in normal and BPH but did not show the possibility of its expression in pathological prostate or its role in the pathogenesis of different pathological conditions of the prostate [22]. We utilize Ub-antibody for localization of Ub in abnormal specimens from human prostate gland and correlate the findings with different pathological conditions of the prostate.

Materials and Methods

Prostatic tissues were taken from patients (aged 64–80 years) with benign prostatic hyperplasia (21 cases) and prostatic adenocarcinoma (7 cases) through transurethral or suprapubic resection. Normal prostatic tissues were taken from corps by autopsy (12 cases). Specimens were clinically and histopathologically diagnosed. The procedures followed for obtaining all specimens were in accordance with the guidelines of the ethical committee of the Faculty of Medicine at Jordan University of Science and Technology. All tissues were fixed in SUSA fluid consisting of formalin, mercuric chloride and glacial acetic acid. Tissues then were routinely processed and embedded in paraffin.

Five μm thick sections were immunostained by avidin-biotin-peroxidase method. In order to abolish endogenous peroxidase activity, sections were incubated for 30 min. in 0.3% hydrogen peroxide in PBS. The sections were incubated overnight at 4°C with the rabbit polyclonal Ub-antibody (Segma MO, USA) at 1:400 dilution, and then incubated for 30 min with biotinylated goat anti-rabbit immunoglobulin as a secondary antibody (Dako) diluted 1:200. Subsequently, they were incubated for 30 min. with avidin-biotinylated peroxidase complex (Dako) diluted 1:100 in PBS. Visualization of the reaction was performed using 3.3’ diamino benzidine tetrahydrochloridehydroden peroxidase as a chromogen in Tris buffer at pH 7.6 for 5 min. Between each step, slides were washed 3 times (5 min. each) with PBS. The sections were counter stained, dehydrated and mounted.

Specificity of the reaction was tested by incubating sections with either preabsorbed primary antibody with excess antigen for 48 hours or with buffer, from which the primary antibody has been deleted, followed by incubation otherwise as usual. Slides were photographed using Olympus photomicroscope (BH2-RFCA).

Results

All prostatic tissues from patients with benign prostatic hyperplasia and adenocarcinoma and from the corps showed a positive reaction for Ub, but with variation in the distribution and intensity of the reaction product. Figure 1 showed a positive reaction for Ub in normal prostatic tissues. The reaction was confined to the epithelium but not the lumen of the acini or the connective tissue. The reaction was very mild in the nuclei and the cytoplasm as well. Both the cytoplasm and the nuclei of epithelial cells lacked uniformity in reaction.

Figure 2 showed a positive reaction for Ub in prostatic tissues from patients with benign prostatic hyperplasia. The reaction was confined to the epithelium and connective tissue as well. The reaction was most intense in the nuclei, while the cytoplasm showed a mild reaction. In contrast, the connective tissue showed heterogeneity in reaction product. Both cytoplasm and nuclei of epithelial cells lacked uniformity in reaction. The secrtery products in the acinar lumen showed a mild reaction for ubiquitin.

Figure 3 showed a positive reaction for Ub in prostatic tissues from patients with prostatic adenocarcinoma. Both the epithelial cells and the connective tissue were stained. The cytoplasm showed the most intense reaction, whereas, the nuclei showed a mild
reaction for Ub in most of the epithelial cells. Some nuclei showed a faint reaction. On the other hand, the secretory products in the acinar lumina and the connective tissue showed a mild reaction for Ub.

Fig. 4 showed hyperplastic prostatic tissue which has been used as a control to validate the technique. No reaction product for Ub, neither in the epithelium nor in the connective tissue, has been detected.

Discussion

All prostatic tissues from normal, BPH, and adenocarcinoma showed a positive immunoreactivity for Ub with a variation in the distribution and intensity of the immunoreactivities. The variation in Ub distribution and intensity may reflect differences in specific functional response with different pathological conditions. In this study, BPH immunoreactivity was confined to the epithelium and connective tissue as well. This is supported by a study of Lombardo et al., where Ub was identified in BPH homogenate using SDS-PAGE [20]. Ubiquitin in the prostate gland could be the source of Ub in human seminal plasma [19]. On other study, Ub immunostaining was confined to the nuclei and cytoplasm of BPH [22]. Degradation, by Ub, of regulatory proteins in the prostate could trigger the development of BPH. Moreover, it was postulated that the predominance of Ub system component (protein gene product 9.5), in the transition zone of the prostate may be attributed to theogenesis of BPH [29]. This could explain the presence of Ub in the fibromuscular connective tissue as an implicated factor in hypertrophy of smooth muscle fibers in the fibromuscular connective tissue. In this regard, Ub was implicated in the degeneration of insect flight muscle [5] and inclusion body myositis [26] which may lend
support for the presence of Ub in the fibromuscular tissue of the prostate.

We demonstrated intense Ub immunoreactivity in the cytoplasm but not the nuclei of epithelial cells of prostatic adenocarcinoma. Many studies have shown the localization of Ub in malignant tissues. In breast neoplasm, Ub was confined to the cytoplasm but not the nuclei of acinar and ductal cells [13]. In addition, ubiquitin was localized in lung, liver, stomach and pancreatic tumors. The Ub immunoreactivity was distributed uniformly in nuclei and cytoplasm of malignant cells regardless the degree of differentiation or origin of the tumor [12]. The absence of Ub immunoreactivity in many normal tissues and the intense reaction for Ub in their malignant forms [12] raises the question about the role of Ub in malignant transformation. The cytoplasm of the epithelial cells of prostatic adenocarcinoma demonstrated the presence of high amount of Ub which reflects a high rate of translational activity. Since the polyubiquitin gene expression is a cytoprotective phenomenon [8], the enhancement of Ub immunoreactivity in tumor cells may be a kind of stress responses induced by various host defense mechanisms in order to protect the cell. Apart from the stress responses, the enhanced Ub immunoreactivity in tumor cells might be due to the high metabolic/catabolic ration of the tumor vs. normal tissues [12].

The cell cycle and cell proliferation are regulated by a complex pathway composed of cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors [28]. P53, one of the cyclin-dependent kinases inhibitors, induces cell cycle arrest and apoptosis and simultaneously stimulates P21 (cyclin dependent kinase inhibitor) which results in G_1 and G_2 arrest. [35]. The role of Ub in P53 proteolytic degradation has been documented [24] and oncoproteins promote malignant transformation.

Figure 3. Photograph of prostatic adenocarcinoma of human prostate gland. Portions of prostatic acini are shown. Intense reaction for ubiquitin was shown in the cytoplasm of the lining epithelium (black arrows) while the nuclei showed a mild reaction for ubiquitin (white arrows). Connective tissue (CT) and the acinar lumen (L) show mild reaction for ubiquitin.

Figure 4. Photograph of the benign prostatic hyperplasia of human prostate gland. Portions of prostatic acini are shown. Control section that has been treated with buffer from which the anti-ubiquitin has been deleted. The cytoplasm (arrows) and nuclei (arrow heads) of the lining epithelium as well as the connective tissue (CT) show a negative reaction for ubiquitin.
in HPV-transformed cervical cell line by stimulating Ub-dependent degradation of P53 [30]. 

On the other hand, Ub degradation of P27, a cell cycle inhibitor, was implicated in the development of prostatic adenocarcinoma. This was associated with a concomitant high amount of Ub-ligase Skp2 levels [2]. A recent study has suggested the involvement of Ub in the pathogenesis of prostatic adenocarcinoma by degradation of proteins involved in growth inhibition or apoptosis of cancer cells [16].

Thus, accumulation of neoplastic cell specific ubiquitinated proteins might be one of the explanations for the enhanced Ub immunoreactivity in prostatic adenocarcinoma.

Bortezomib, formerly known as (PS-341, LDP-341 and MLM341), is a potent anti-cancer agent against a variety of tumors including prostate cancer is a selective proteasome inhibitor which might supports the implication of Ub in prostate cancer pathogenesis [1]. On the other hand, the induction of S-phase arrest and apoptosis in LNCaP and PC-3 prostate carcinoma cells by a novel retinoid CD437 [18] was regulated by ubiquitin-mediated pathway [6].

Although it is too early to conclude the relationship between the level of Ub and some physiological states of cancerous or hypertrophied prostate, the differences found in Ub distribution could be used as a cytological parameter in the diagnosis and prognosis of disease states of the prostate.

In this regard, the intensity of prostatic acid phosphatase was used to differentiate between normal, BPH and adenocarcinoma of the prostate [33]. Other study has used the prostatic specific antigen in localization, staging and prognosis of prostatic adenocarcinoma [25]. On the other hand, overexpression of Skp2 (Ub ligase) and loss of P27 (cyclin dependent kinase) by Ub system degradation are strongly associated with poor prognosis and may thus be used as a prognostic marker for colorectal carcinoma [31] and prostatic adenocarcinoma [36], respectively. Although this study did not determine the Ub expression as an independent predictor of the pathological states of the prostate, Ub expression is of significant interest and warrants further investigation as a potential marker for cell proliferation in the prostate.

These preliminary data may set the bases for further studies which correlate Ub distribution with different stages and differentiation of prostatic tumors.

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